INNOVATIVE CEROSOMES AS CARRIERS FOR AN ENHANCED DERMATOSES' THERAPY

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ABSTRACT

Dermatoses are skin diseases caused by lowered level of lipids in the intercellular lipid matrix of skin's uppermost layer *stratum corneum*. A promising therapy is the application of liposomally formulated skin lipids (ceramides, cholesterol, fatty acids). These so called cerosomes proved very effective in disrupted skin barrier repair; however, they aim primarily on a mechanical surface restoration. For this reason an interesting idea is to combine the cerosomes and anti-inflammatory drugs (such as hydrocortisone) used for the treatment of pathophysiological processes in the skin. Unfortunately, liposomes generally show low drug encapsulation rates, hence they would not be very effective carriers. On the other hand, great encapsulation effectivity can be found in lipid nanocapsules (LNCs). We therefore aimed to create a combined system cerosomes-LNCs that would ensure the skin barrier restoration thanks to the ceramides and simultaneously effectively deliver hydrocortisone as a representative of anti-inflammatory drugs into lower layers of the skin. In this work, we characterized the formulations by dynamic light scattering and transmission electron microscopy to assess their size and morphology.

Keywords: atopic dermatitis, ceramides, cerosomes, lipid nanocapsules, hydrocortisone

INTRODUCTION

Human skin is the most effective barrier protecting our bodies from xenobiotics, UV light or bacteria. It ensures the proper thermoregulation and limits the water loss. All these vital properties are possible thanks mainly to the uppermost layer of the skin – *stratum corneum* (SC) and its unique composition. Its main building blocks are the skin cells keratinocytes that are connected by a complex intercellular lipid matrix. This lipid matrix consists of ceramides (40–50%), fatty acids (15–25%), cholesterol (20–25%) and cholesterol sulfate (5–10 %) (Harding, 2004).

Skin, as any other organ, can be affected by a disease that changes or limits its basic functions. The most common ones one can encounter nowadays are atopic dermatitis, or generally dermatoses, or psoriasis. These diseases are accompanied by loss of skin hydration, dryness and elevated permeation rates of xenobiotics. The affected sites are reddish and itchy, which can lead to the necessity to scratch the area and therefore to the probability to carry an infection into the deeper layers of the skin and to further spread of the disease. One of the common signs of the diseases compromising the skin barrier is the change in the SC lipid matrix composition. The most notable change here is a depletion of ceramides and long chain fatty acids (Shao et al., 2016; Wolf & Wolf, 2012).

The typical treatment of skin diseases is the application of ointments and creams, whose task is to ensure sufficient skin hydration and provide a healing environment. The most widely used therapy is application of corticosteroids and calcineurin inhibitors. These drugs are however administered in a form of suspension or dispersion, which are forms that cannot ensure the best biological availability of topically applied drugs. For this reason, we focus on the development of an alternative therapy concerning the direct application of the skin lipids, namely ceramides that help to restore the disrupted skin barrier properties.

Currently, we have a very effective liposomal system containing skin lipids with a composition imitating the SC lipid matrix – so called cerosomes. These cerosomes

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are able to effectively restore chemically disrupted skin barrier (Vovesná, Zhigunov, Balouch, & Zbytovská, 2021). However, their effect is directed only to the outer surface disease manifestations. It is therefore vital to enhance this system and target even the pathophysiological processes in the deeper skin layers. For this purpose, we already have an experience with hydrocortisone-loaded cerosomes. Nevertheless, the encapsulation efficiency of cerosomes was not ideal and the system would not be as effective as needed. Typically for a topical administration of drugs, we use another carriers – lipid nanocapsules (LNCs). These nanoparticles are stable for a long time with great capability to encapsulate wide variety of drugs, including HC. We also know, that LNCs help to concentrate HC into SC and epidermis; however, they do not restore the skin barrier.

For this reason we aim to create a combined system of cerosomes and LNCs with encapsulated anti-inflammatory drug (eg. hydrocortisone). We will characterize the morphology of prepared particles and ultimately test their efficacy in disrupted skin repair and HC administration.

RESEARCH CONCEPT

Preparation of cerosomes-LNC system

The cerosomes are prepared by modified method of thin lipid film hydration. At first, all the lipids, ceramides 3 and 6, cholesterol and stearic acid are weighed in a round bottom flask and dissolved in a chloroform : methanol mixture. Hydrocortisone (HC) or HC-butyrate (HC-B) is added to this mixture as well. The ratio of these lipids and the drug is equimolar to best simulate the environment in the lipid matrix and to introduce sufficient amount of the drug into the formulation (Table 1). The solvents are then evaporated on a rotary evaporator until a thin film is formed on the walls of the flask. This film is further hydrated by phosphate buffered saline (PBS) solution with 10 % urea addition (PBS+U). The flask is alternatively placed in a heater on 95 °C and shook on a vortex until all the lipids from the flask walls are dissolved. Next, LNCs are prepared by the phase inversion temperature method (Heurtault, Saulnier, Pech, Proust, & Benoit, 2002). An MCT oil (Miglyol 812 N), nonionic (Kolliphor HS 15) and amphoteric (Phospholipon 90G) surfactants are weighed in a beaker. HC or HC-B are also added to this mixture. PBS with 10 % addition of urea (PBS+U) is added and the whole is placed on a heater (Table 1). The mixture is heated three times in the range from 50 to 85 °C. After the third heating cycle, the mixture is diluted with ice cold PBS+U. The cold shock induces the nanoparticle formation and also the encapsulation of the drug.

Table 1: Composition of prepared nanoparticular systems.

Cerosomes			LNCs	
Compound	**wt%**	**wt%**	**Compound**	**wt%**
Ceramide 3	0.18	0.17	Miglyol	30.0
Ceramide 6	0.18	0.18	Kolliphor	20.0
Cholesterol	0.22	0.26	Phospholipon	1.5
Stearic acid	0.24	0.23	HC, HC-B	0.75
HC	0.18		PBS+U	15.0
HC-B		0.17	PBS+U (0°C)	32.75
Urea	10.0	10.0		
PBS	89.0	89.0		

To combine these two systems, we took two approaches. One is to add the LNCs to the cerosomes. A freshly prepared batch of LNCs was added onto the thin lipid cerosomal film. The film was then hydrated and vortexed the same way as described above. The second variation of the preparation is to add the cerosomes to the LNCs. At first, cerosomes were prepared as described and then they were added to the LNC preparation instead the sole PBS+U.

Particle characterization

We characterized our nanoparticulate systems by dynamic light scattering (DLS) in order to get the information about the size of present particles. We describe our systems by number mean values of the size when we show characteristics of sole cerosomes or LNCs. Then to describe the combined system, we use volume mean values of the size for the reason that it is crucial to see both types of the particles largely differing in their sizes.

We also established the encapsulation efficiency (EE) of cerosomes, LNCs and cerosomes-LNCs for hydrocortisone and hydrocortisone-butyrate. The EE was determined by ultrafiltration of a sample through Centrisart 20 kDa MWCO filtration units and subsequent analysis of the supernatant.

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RESULTS AND DISCUSSION

At first, we prepared and characterized both systems (cerosomes and LNCs) respectively to find the maximal content of the drug (HC, HC-B) and other limits for both formulations. Loading cerosomes with a drug like HC or HC-B did not pose any challenge in terms of preparation conditions. It was possible to hydrate the lipid films containing both drugs in normal hydrating time and temperature (6 h, 95 °C). The resulting liposomal formulation was of a smooth white appearance without any visible large particles, crystals or aggregates. The particles were stored in a dark place at laboratory temperature in order to minimize influences on the particle degradation. Liposomes prepared by film hydration method are large multilamellar vehicles with quite notable variations in size leading to higher values of polydispersity index (PDI). Our prepared cerosomes showed following characteristics: HC-cerosomes were of 651 ± 25 nm in size with PDI = 0.37 ± 0.12. HC-B-cerosomes did not differ significantly; they contained particles with size of 773 ± 45 nm, PDI = 0.45 ± 0.10. As the particle size is concerned, drug-loaded cerosomes were in every case smaller than cerosomes prepared without an API (active pharmaceutical ingredient). To show the difference, we can compare CER3+CER6 type of cerosomes prepared in our previous work (Vovesná et al., 2021). There we show the cerosomes to be 1196 ± 238 nm large with PDI of 0.15 ± 0.02. That is notably bigger size than HC or HC-B loaded cerosomes with the same composition. We assume that this fact can be ascribed to the molecular shape of both corticoids. The base structure of the sterol core of HC and HC-B is similar to the structure of cholesterol; therefore, HC could have the tendency to incorporate itself into the bilayered mixed structure of the cerosomes, opposed to more common adherence or bonding onto or into the liposomes. For this reason, we also chose the total content of HC and HC-B to be equimolar with all other components. Ultimately, the lipids form 1 % of the total formulation and HC or HC-B stand for approximately 0.18 % of total. The cerosomes as other liposomes do not possess great encapsulating capacity. That is also the main reason for this work on a combined system. In our case the encapsulation efficiency (EE) for HC was 25.3 ± 1.4 % and drug load (DL) 0.3 %. For HC-B these values were similar: $EE_{HCB,cersosomes}$ = 20.6 ± 0.9 and $DL_{HCB,cerosomes}$ = 0.2 ± 0.1 %. The encapsulation values around 20 % are in the expected range. DL usually reaches values < 10 % and for liposomes, these values are as always low.

The second formulation that forms the combination are the LNCs. The composition of the LNCs in this work was optimized to hold the highest amount of HC or HC-B possible without influencing the particle stability. The maximal content of the corticoids in LNCs is 0.75 % of the total formulation for both APIs. The size of empty LNC particles prepared with this composition (Table 1) is 101 ± 3 nm with PDI = 0.06. This size is comparable to other LNCs with quite same composition prepared in our laboratory (Pytlíková, 2020) that were sized circa 115 nm with PDI of 0.07. The introduction of an API into this system changed its characteristics only minimally. HC-LNC contained particles of 105 ± 2 nm, PDI = 0.08 and HC-B-LNC were sized 113 ± 1 nm, PDI = 0.09. These changes are very slight and can even be ascribed to variation in preparation and measurements. As was proposed, LNCs have great encapsulation ability for vast variety of drugs. Our system is not an exception, even though the selected corticoids are generally quite inert and insoluble. The EE for hydrocortisone was as high as 87.6 ± 3.1 % and DL was 1.0 ± 0.1 %. HC-B-LNCs encapsulated the drug by 85.8 ± 1.7 % with DL of 1.0 ± 0.1 % also. Additionally, all these characteristics did not change significantly during 2 weeks storage time, implying that HC and HC-B loaded LNCs are stable system capable to incorporate notable amount of the APIs.

For the last part of this work, we combined the two described systems in order to create a complex renewing and healing treatment for dermatitic conditions. The first approach was to use the HC(B)-LNCs as the aqueous phase for the HC(B)-cerosomes. However, the heating cycles at 95 °C seemed to disrupt and break the constitution of the LNCs due to such high applied heat. The DLS measurement revealed only particles sized around 1 μm, which suggests the presence of liposomal structures, but not the smaller LNC ones. It was not possible to lower the hydrating temperature for the ceramides would not hydrate in the needed time. We therefore applied another method for this combination. LNCs were again added onto the lipid film and the whole flask was placed in a water bath set at 40 °C and the mixture was intensely mixed overnight to simulate a kind of high shear homogenization conditions. After this time, the lipid film was completely hydrated from the flask walls; however, there could still be seen larger crystals of incompletely dissolved components of the formulation. The second approach to the preparation was to use the HC(B)-cerosomes as the aqueous phase of the HC(B)-LNCs. Their preparation process was not hindered by the exchange of PBS+U for the cerosomes

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and was completed as described in the methods section. The resulting formulation was homogeneous, opalesque without visible particles. The DLS measurement confirmed the presence of two sizes of particles if evaluated by volume mean. For HC variation the first and larger peak was at 98 ± 2 nm at should belong to the LNC part of the combination. The second peak was visibly smaller and positioned at 835 ± 11 nm, which accords with the size of cerosomes. For HC-B the result was really similar. It was possible to obtain a formulation with two distinct sizes of 116 ± 5 nm and 956 ± 45 nm belonging to LNCs and cerosomes respectively. The EE and DL for this system are yet to be determined. Nevertheless, from the fact that the formulations did not contain any visible undissolved crystals, which it otherwise would if the HC and HC-B were not dissolved, we can expect the encapsulation rates to be at least as good as for the LNCs, but with the total amount of the API higher than in both sole formulations.

CONCLUSIONS

In this work we focused on a preparation of nanoparticulate systems for topical delivery of hydrocortisone and hydrocortisone-butyrate. It was possible to prepare lipid nanocapsules containing 0.75 % of HC or HC-B with 85 % encapsulation efficiency. Next, we successfully prepared cerosomes mimicking the composition of *stratum corneum* lipid matrix loaded with 0.18 % of HC and HC-B. In order to enhance the efficiency of dermatitic conditions treatment, we successfully combined the two systems and characterized it for the presence of both formulations. We propose that such complex formulation has high potential in disrupted skin barrier treatment.

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SUBSCRIPT

API	Active pharmaceutical ingredient
DLS	Dynamic light scattering
EE	Encapsulation efficiency
HC	Hydrocortisone
LNC	Lipid nanocapsule
PBS	Phosphate buffered saline
SC	*Stratum corneum*
TEM	Transmission electron microscopy

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